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Acid gelation of raw and reconstituted spray-dried dromedary milk: A dynamic approach of gel structuring

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ABSTRACT

Dynamic oscillatory rheology and scanning electron microscopy (SEM) were used to further understand the formation of acid milk gels obtained from raw and reconstituted spray-dried dromedary milk. The influence of acidification temperature, concentration of acidifier (glucono-δ-lactone; GDL) and milk fat content on the rheological parameters of milk gelation were firstly investigated. Our results confirmed the poor ability of dromedary milk to be processed into acidic gels. Under optimised conditions (45 °C, 2.25% GDL), the gel point of dromedary milk occurred at a lower pH (4.46) than that of bovine milk (5.06). The resulting gel obtained after 3 h acidification had a storage modulus (G′) 35 times lower than that obtained from bovine milk. SEM observations of dromedary and bovine milk during acidification highlighted many important differences in the implementation of the microstructure of acid gels. These fundamental differences are discussed based on the literature knowledge of cows' milk acid gelation.

1. Introduction

Milk is a complex food matrix that is composed of proteins, lipids, sugar (lactose), minerals and trace compounds including vitamins (Walstra & Jenness, 1984). Cow milk is the most consumed milk worldwide with around 635 million tons produced in 2013. However, in some arid and semi-arid areas of the world, dromedary milk is the primary source of dairy products. World dromedary milk production in 2013 was over 2900 thousand tons, of which only 1400 tons were produced in Tunisia (FAOSTAT, 2013). The overall general composition of cow and dromedary milk are similar (Elagamy, 2000). However, dromedary milk is higher in β-casein, and lower in κ-casein. Dromedary milk also lacks β-lactoglobulin (Kappeler, Farah, & Puhan, 1998; Merin et al., 2001).

There has been considerable interest in the last few decades in dromedary milk derivative products as means of conserving the milk. Traditionally, conservation involves acid coagulation or microbiological fermentation, depending on indigenous culture and habits (Attia, Kherouatou, & Dhouib, 2001; El Zubeir, Abdala, & Owni, 2005). For example, as reported by Abdelgadir, Ahmed, and Dirar (1998), 'Garris' in Sudan is prepared by mixing the dromedary milk with soured acid products in large bags or containers. Likewise, in Kenya, 'Suusac' is obtained after one to two days of fresh dromedary milk fermentation in a pre-smoked gourd (Lore, Mbugua, & Wangoh, 2005). Other dromedary milk derivatives are also produced, mainly butter (Berhe, Seifu, & Kurtu, 2013; Farah, Streiff, & Bachmann, 1989) and cheese (El Zubeir & Jabreel, 2008; Mehaia, 2006). During the conventional cheese-making process, dromedary milk coagulates slower than cows' milk and has a weaker coagulum (Ramet, 2001). However, comparable dromedary cheese yields are now possible thanks to technological advances including thermophilic starters and Chy-Max® (Konuspayeva et al., 2017).

Dromedary milk yoghurt has poor sensorial profiles if made without adding gelling additives. It has a low viscosity, an instable coagulum and a higher susceptibility to syneresis than cow milk yoghurt (Hashim, Khalil, & Habib, 2009; Ibrahim & Khalifa, 2015). Relatively few studies have investigated the biochemical mechanism of dromedary milk acid gelation through micellar fraction changes during acidification. Attia, Kherouatou, Nasri, and Khorchani (2000b) reported that the physicochemical composition of dromedary milk, the rapid dissociation of casein micelles, and the slow rate of new interactions (protein/protein and protein/water) during acidification are likely the main factors responsible for the production of a weak coagulum at acid pH.

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Acid coagulation of cow milk has been extensively studied and described in the literature. The milk acidification destabilises casein micelles and reduces their charge. It dissolves some insoluble calcium phosphate crosslinks and changes protein:protein interactions. As electrostatic repulsion is reduced protein aggregates form and ultimately gel. Acid-induced milk gels get firmer from casein bond formation. It was reported that the physicochemical environmental factors such as milk proteins composition, ionic strength and mineral content, especially calcium, had an important impact on acid gel network development (Morand, Dekkari, Guyomarc'h, & Famelart, 2012; Salvatore, Pirisi, & Corredig, 2011).

The purpose of this study was to better understand the properties of dromedary milk with an eye to further development as a food stuff that can be stored and used in arid countries. To create a controlled study with comparisons, we evaluated the characteristics of skimmed dromedary milk prior to acidification and for a cross-species comparison we also studied reconstituted skimmed cows' milk. The influence of different physico-chemical parameters (temperature, acidifier concentration and dromedary milk fat content) on the acid gelation of dromedary milk was investigated, as well as the gel microstructure during chemical acidification. We also tested the influence of spay drying of dromedary milk on the acid gelation of reconstituted milk.

2. Materials and methods

2.1. Milk samples

Dromedary (Ardhaoui variety) and cow (Holstein breed) milk were collected from two separate herds in southern Tunisia (Gabes and Sfax governorates, respectively) and transported to the laboratory one to 2 h after milking. Milk samples were immediately stabilised against microbiological development with addition of 0.02% (w/w) of sodium azide and stored at −20 °C until use. Cow and dromedary milk samples were skimmed using 1 or 3 successive centrifugations (2000 × *g*, 15 min, 5 °C), respectively, before freezing. The final fat content of skimmed dromedary milk (SDM) and skimmed cow milk (SCM) was very close and equal to 1.05 ± 0.21 g L^{-1} and 1 g L^{-1}, respectively. Both types of skimmed milk and one litre of whole dromedary milk were rapidly frozen to −20 °C and thawed at 4 °C overnight before experiments. Milk freezing was necessary to ensure its preservation for several months. No particle or aggregate was observed in milk samples after thawing.

2.2. Dromedary and cow milk powder production

Skimmed dromedary and cow milks were spray-dried using a mini spray dryer (Büchi, B-290, Flawil, Switzerland). Inlet drying temperature was set to 190 °C and the feed rate was optimised (0.6 L h^{-1}) to maintain an outlet drying temperature of 90 °C. The powders were then stored at 4 °C in sterile plastic pots away from light and humidity.

Reconstituted skimmed dromedary milk (RSDM) or reconstituted skimmed cow milk (RSCM) were obtained by rehydration of respective powders with ultrapure water to obtain the same protein content as in the skimmed dromedary milk (SDM). The mixtures were stirred at 580 rpm for 30 min at room temperature. The reconstituted milk samples were stored at 4 °C overnight and then warmed up for 1 h at 40 °C before further use.

2.3. Protein content determination

The bicinchoninic acid assay (BCA, G-Biosciences, Geno Technology, St. Louis, MO, USA) was used to determine the protein content of dromedary and cow milks.

After dilution (100 fold in ultrapure water), 1 mL of the reagent solution (Cu^{2+} and BCA in 1:50, v/v) was added to 50 μL of each milk sample. The mixture was incubated at 37 °C for 30 min in total darkness. Prior to reading the absorbance at 562 nm (quartz cell), the samples were cooled to room temperature. Bovine serum albumin (BSA) was used as the standard to make a calibration curve, covering protein concentration from 0 to 2 g L^{-1}.

2.4. Protein identification and quantification

Identification and quantification of dromedary or cow milk proteins (total or micellar fraction) were performed by reversed phase high performance liquid chromatography (RP-HPLC) using an HPLC unit (Waters, Milford, MA, USA).

Micellar fractions were obtained after centrifugation of skimmed milk at 20,000 × *g* for 15 min at 20 °C. Pellets containing the casein micelles fraction were suspended in 10 mL of a simulated milk ultrafiltrate solution (SMUF) (1:10, v/v; Jenness & Koops, 1962) and filtered through a cellulose acetate membrane (pore diameter 0.8 μm, Sartorius, Göttingen, Germany). To identify the β-casein peak, part of the micellar fraction was stored at 4 °C overnight and a second centrifugation at 20,000 × *g* for 15 min at 4 °C was performed. At this temperature, the β-casein migrates from the casein micelle into the soluble phase. All fractions were prepared as described by Bobe, Beitz, Freeman, and Lindberg (1998) for chromatography analysis.

Before sample injection (20 μL), the pumps and the column (Interchrom column, C18, length 250 mm; diameter 4.6 mm; particle size 17.10 μm, pore size 10 nm, Interchim, Montluçon, France) were flushed with water or a buffer solution composed of acetonitrile, water and trifluoroacetic acid (TFA) (1:9:0.01, by vol; eluant A) for 30 min at 40 °C with a flow rate of 1.2 mL min^{-1}. After injection, a gradient elution (to acetonitrile, water, TFA, 9:1:0.01, by vol; eluant B) was applied as described by Bobe et al. (1998).

2.5. Milk granulometric analysis

Size distributions of dromedary casein micelles (obtained as described in section 2.4) were determined by dynamic light scattering using a Nanosizer (Nano-ZS, Malvern Instruments Ltd, Malvern, UK) equipped with He-Ne laser light (λ = 633 nm) and a photodiode detector with a detection angle of 173°. The Nanosizer cell temperature was set and maintained at 25 °C during all measurements. Assuming that casein micelle particles are spherical and monodisperse, their hydrodynamic diameter was calculated following the Stokes–Einstein relation using the average diffusion coefficients that were determined by the cumulant method.

2.6. Acid gelation monitoring

Milk samples were equilibrated in a water bath until the desired temperature was reached (15, 30 or 45 °C). The acidification of milk samples was carried out by adding defined amounts of glucono-δ-lactone (GDL; Merck, Darmstadt, Germany) to reach concentrations between 1.75 and 2.5% (w/w). Milk samples were then stirred for 1 min and divided into two 20 mL parts: one for the acidification monitoring and the other one for rheology measurements. When added to milk, the GDL gradually dissociates into gluconic acid, which induces a progressive acidification of milk that mimics the action of lactic bacteria during milk fermentation.

2.6.1. Acidification kinetics

Milk samples containing GDL were immediately placed in a water bath at 15, 30 or 45 °C and pH values were recorded over 4 h using a pH-meter (C831, Consort, Turnhout, Belgium) connected to

a data acquisition system taking one measure every 15 s. For each experiment, at least three replicates were performed.

2.6.2. Dynamic oscillatory rheology

Rheological measurements were carried out using a dynamic rheometer (MCR-300, Anton Paar, Graz, Austria) equipped with a coaxial cylinder measuring system (CC27) and a Peltier system, which maintains a constant temperature throughout the gelation process. Milk samples with GDL were immediately placed in the cup of the rheometer and dynamic measurements (storage modulus, G′; loss modulus, G″; damping factor, tan δ) were recorded for 3 h at a frequency of 1 Hz and a strain of 1% (with respect of linear viscoelastic region). For each experiment, three replicates were performed. The gel point was experimentally determined as the point where the value of G′ was equal to the value of G″, which defines the beginning of gelation process.

2.6.3. Scanning electron microscopy

To carry out scanning electron microscopy observations of milk during acidification, milk samples were fixed on inorganic filtration membranes (Anodisc 25, scanning electron microscopy grade, 2 cm in diameter, average pore size 0.2 μm, Whatman, Maidstone, UK). For this, membranes were immersed in milk samples just after the addition of GDL and subsequently removed from the milk during acidification at different pHs: initial pH; pH 5.5; gelling pH and pH 4 (final pH). Then, milk samples that were fixed on the membranes were immediately dehydrated through a graded series of ethanol/water baths (25, 50, 70, 80, 90, 96, 100%, v/v; 5 min each). Dehydration of samples was completed using a CO_2 critical point dryer (CPD 030 Bal-Tec, Balzers, Liechtenstein). Metallised samples were then observed using a Hitachi S-4800 high-resolution scanning electron microscope (Tokyo, Japan) at two magnifications (10,000× and 25,000×).

2.7. Statistical analysis

Statistical analysis was performed using Statistica 10 software (StatSoft, Inc., Oklahoma, USA, 2011) following the one-way analysis of variance (ANOVA) and Fisher's least significant difference analysis ($p < 0.05$). Results are presented as the mean ± standard deviation.

3. Results and discussion

3.1. Physico-chemical characteristics of milk samples

The physico-chemical characteristics of dromedary and cow milk used in the present study are summarised in Table 1. Skimmed dromedary milk (SDM) presents a significant lower pH value (6.35) than reconstituted skimmed cow (RSCM) and dromedary milk (RSDM) (pH = 6.55). All protein fractions were similar ($p > 0.05$) for the three types of milk (Table 1). However, analysis of casein composition of dromedary milk shows significant differences as compared with cow milk. The micellar fraction of dromedary milk had a higher proportion of β-casein and a lower proportion of κ-casein than cow milk, whereas both types of milk have a similar proportion of α-casein. Furthermore, the mean hydrodynamic diameters of casein micelles from skimmed and reconstituted dromedary milks were 298 ± 9 and 442 ± 12 nm, respectively, and are significantly higher than those of cow casein micelles (240 ± 9) (Table 1). These results are in agreement with those of Kherouatou, Nasri, and Attia (2003) who also found larger casein micelles in dromedary milk than in cow milk. The higher casein micelle diameter of reconstituted compared with non-reconstituted dromedary milks is likely due to interactions between caseins micelle and whey proteins that are induced by heat during spray drying. In the same way, diameters of casein micelles from reconstituted cow milk (~240 nm, Table 1) are also higher than those reported for raw cow milk (179–204 nm; Glantz et al., 2010).

3.2. Influence of physico-chemical parameters on the acid gelation of dromedary milk

The influence of different physico-chemical parameters (GDL concentration, fat content, or temperature) on acidification kinetics and rheological properties of dromedary milk are presented in Table 2.

3.2.1. Influence of glucono-δ-lactone concentration

The influence of GDL concentration was carried out at 30 °C using whole dromedary milk (Table 2). Increasing the GDL concentration from 1.75 to 2.5% (w/w) accelerated the rate of acidification and led to a lower final pH (4.16 compared with 3.75, respectively). However, the GDL concentration did not affect the pH (≈4.4) or the rheological properties (storage modulus, G′ ≈ 0.24 Pa) of the gel point (Table 2).

Furthermore, even though dromedary milk made extremely weak acid-gels, some trends were evident. For example, higher GDL concentrations of 2.25 or 2.5% led to significantly firmer gels than did lower concentrations (1.75 or 2%). This was equally true just 1 h after the gelation point was reached as it was at the final 3 h timepoint. Therefore, the rate of acidification of dromedary milk has no impact on the characteristics of the gel point (pH, G′) at 30 °C. However, the rate of acidification of dromedary milk does influence the final pH of gels and their rheological properties.

Similar findings were observed for cow milk (Lucey, van Vliet, Grolle, Geurts, & Walstra, 1997) and whey proteins acid gelation (Cavallieri & da Cunha, 2008). These authors reported that the acidification rate with GDL did not significantly affect the rheological properties of acid gel at the beginning of gelation process. However, it had a significant effect on the final protein network

Table 1
Physico-chemical characteristics of skim and reconstituted skimmed dromedary milk and reconstituted skimmed cow milk.[a]

Parameter	SDM	RSDM	RSCM
pH	6.35 ± 0.03[a]	6.55 ± 0.004[b]	6.55 ± 0.001[b]
Total proteins (g L^{-1})	26.7 ± 0.7[a]	25.4 ± 0.8[a]	26.5 ± 1.3[a]
Micellar fraction	19.0 ± 1.2[a]	18.5 ± 1.2[a]	19.3 ± 1.6[a]
Soluble fraction	7.8 ± 0.6[a]	5.9 ± 0.4[b]	7.1 ± 0.4[b]
Casein (%)			
α-casein	34.9	nd	34.4
β-casein	47.1	nd	29.5
κ-casein	3.5	nd	9.4
Casein micelle mean diameter (nm)	298 ± 9	442 ± 12	240 ± 6

[a] Abbreviations are: SDM, skimmed dromedary milk; RSDM, reconstituted SDM; RSCM, reconstituted skimmed cow milk; MF, micellar fraction. Values for caseins are percentages relative to total caseins. Values in a row followed by different superscript letters are significantly different ($p < 0.05$); nd, not determined.

Table 2
Influence of glucono-δ-lactone (GDL) concentration, fat content, and temperature of acidification on the characteristic parameters of acid gelation of dromedary milk.[a]

Influence	Gel point			1 h after gel point		After 3 h acidification	
	Time (s)	pH	G′ (Pa)	pH	G′ (Pa)	pH	G′ (Pa)
GDL concentration (%, w/w)							
1.75	6722 ± 268^{a}	4.45 ± 0.04^{a}	0.24 ± 0.01^{a}	4.19 ± 0.04^{a}	1.52 ± 0.38^{a}	4.16 ± 0.01^{a}	1.54 ± 0.28^{a}
2	5540 ± 447^{b}	4.39 ± 0.12^{a}	0.25 ± 0.02^{a}	4.15 ± 0.07^{a}	1.6 ± 0.25^{a}	4.04 ± 0.07^{b}	1.82 ± 0.22^{a}
2.25	4222 ± 205^{c}	4.46 ± 0.03^{a}	0.24 ± 0.02^{a}	3.99 ± 0.08^{a}	2.63 ± 0.45^{b}	3.87 ± 0.03^{c}	3.16 ± 0.10^{b}
2.5	3519 ± 199^{d}	4.38 ± 0.15^{a}	0.24 ± 0.03^{a}	3.91 ± 0.14^{b}	2.19 ± 0.5^{b}	3.75 ± 0.03^{d}	2.69 ± 0.55^{b}
Fat content (g L^{-1})							
21.1 ± 0.1	4222 ± 205^{a}	4.46 ± 0.03^{a}	0.24 ± 0.02^{a}	3.99 ± 0.08^{a}	2.63 ± 0.45^{a}	3.87 ± 0.03^{a}	3.16 ± 0.10^{a}
1.05 ± 0.2	5340 ± 173^{b}	4.51 ± 0.05^{b}	0.23 ± 0.03^{b}	4.20 ± 0.03^{b}	1.12 ± 0.06^{b}	4.10 ± 0.01^{b}	1.41 ± 0.06^{b}
Temperature (°C)							
15	9710 ± 206^{a}	4.59 ± 0.02^{a}	0.25 ± 0.03^{a}	nd	nd	4.50 ± 0.00^{a}	0.60 ± 0.02^{a}
30	5340 ± 173^{b}	4.51 ± 0.05^{a,b}	0.23 ± 0.03^{a}	4.20 ± 0.03^{a}	1.12 ± 0.06^{a}	4.10 ± 0.01^{b}	1.41 ± 0.06^{b}
45	2225 ± 338^{c}	4.47 ± 0.04^{b}	0.20 ± 0.03^{a}	3.98 ± 0.03^{b}	0.92 ± 0.2^{a}	3.83 ± 0.02^{c}	1.74 ± 0.44^{b}

[a] Influence of GDL concentration was investigated at 30 °C with whole dromedary milk; influence of fat content was investigated at 30 °C at a GDL concentration of 2.25% (w/w); Influence of temperature was investigated at a GDL concentration of 2.25% (w/w) using skimmed dromedary milk. Values in a column within an influencer set followed by different superscript letters are significantly different ($p < 0.05$); nd, not determined.

strength depending of the rearrangement and interactions of proteins after the gel point was reached.

In addition, we found that increasing the GDL concentration from 2.25 to 2.5% significantly increases the initial rate of acidification of dromedary milk but does not further improve the firmness of the final gel (Table 2). These results could be due to the lower final pH reached by the dromedary milk with 2.5% of GDL after 3 h of acidification as compared that reached with 2.25%.

Indeed, several previous studies reported that the strength and stability of cow milk acid-induced gels were affected when the final pH value was lower than the casein micelle isoelectric point (Fetahagić, Maćej, Denin-Đurđević, & Jovanović, 2002; Lakemond & van Vliet, 2008). This suggests that electrostatic repulsion becomes predominant at low pH, destabilising the protein network.

According to these results, we used 2.25% (w/w) as the optimal GDL concentration for the rest of the study.

3.2.2. Influence of fat content

To study the influence of fat content on dromedary milk acid gelation, we acidified whole and skimmed dromedary milks with 2.25% (w/v) of GDL at 30 °C (Table 2). Whole milk became acidic more quickly than skimmed milk. However, both whole and skimmed milks solidified at the same pH of ~4.5 and their corresponding storage modulus G′ values were both very close to 0.24 Pa. After 1 h of acidification from the gel point, whole dromedary milk (G′ = 2.63 ± 0.45 Pa) was over two times as firm as the skimmed milk (G′ = 1.12 ± 0.06 Pa). The positive influence of fat on the rheological properties of acid-gels is well described for cow milk (Lucey, Munro, & Singh, 1999). It was suggested that the fat globules are trapped in the pores of the protein network and interact as fillers within the protein network to strengthen gels (Houzé, Cases, Colas, & Cayot, 2005; Titapiccolo, Corredig, & Alexander, 2011). Other studies highlighted the influence of the size of fat globules on acid gel firmness (Dimitreli, Petridis, Akakiadou, & Chrysalidou, 2014; Ji, Lee, & Anema, 2011). These authors reported that acid gelation of cow and buffalo milk had greater storage modulus G′ when fat globules were smaller. The mean size of fat globules in whole dromedary milk is significantly lower than those in raw cow milk (Attia, Kherouatou, Fakhfakh, Khorchani, & Trigui, 2000a) and this likely explains the positive influence of dromedary milk fat on the rheological properties of acid-gels that we observed (Table 2).

3.2.3. Influence of temperature

Acidification induced by GDL dissociation closely depends on the milk temperature. Here we studied three temperatures (15, 30, 45 °C) for their effect on milk acidification with the optimal GDL concentration of 2.25% (w/v). All experiments were performed on skimmed dromedary milk to avoid interaction between fat and casein micelles during the gelling process (Table 2).

Increasing acidification temperature significantly decreased the time to reach the gel point, from 2.7 h at 15 °C to 37 min at 45 °C (Table 2). There was no significant difference in the pH value at the gel point ($p > 0.05$) between 15 and 30 °C or between 30 and 45 °C. However, the pH of the gel point is slightly, but significantly, lower for an acidification temperature of 15 °C as compared with 45 °C. Furthermore, the temperature of acidification of skimmed dromedary milk (SDM) does not affect the storage modulus value of the gels (Table 2; $p > 0.05$). Hatami, Nejatian, and Mohammadifar (2012) reported that acid gelation temperature of 25, 35 and 45 °C does not affect the gelation pH of cow milk proteins; however, they found that the storage modulus was significantly higher at 25 °C.

Fig. 1 shows a time-course of the storage modulus of dromedary milk over the full 3 h of acidification as a function of pH for the different temperatures of acidification. For the same pH, the corresponding storage modulus of dromedary milk is significantly higher at 15 °C than at 30 or 45 °C. In fact, at 15 °C, the highest final

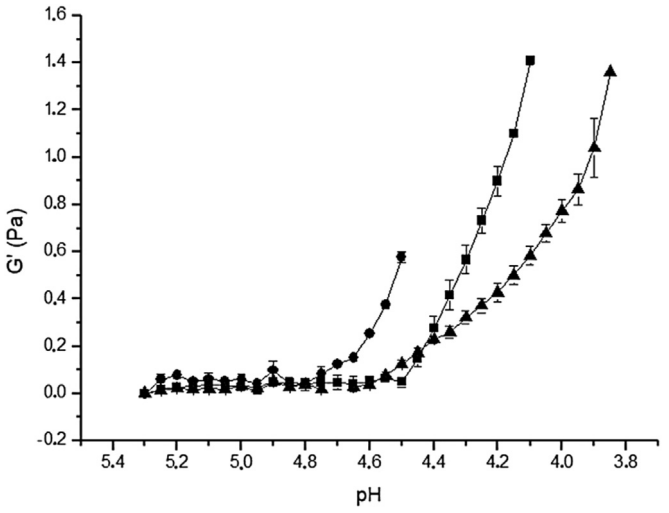

Fig. 1. Influence of the acidification temperature of skimmed dromedary milk on the evolution of storage modulus (G′) as a function of pH. Acidification was carried out with 2.25% (w/w) glucono-δ-lactone at: ●, 15 °C; ■, 30 °C; ▲, 45 °C; data are means ± standard deviation (n = 3).

G′ measured after 3 h of acidification was 0.7 Pa (at a pH value of 4.5). For the same value of pH, the G′ is significantly lower (<0.2 Pa) at 30 or 45 °C and the gel point is not yet reached. These results are in agreement with previous studies carried out with cow milk. Lucey et al. (1997) studied the influence of acidification temperature on rheological properties of acid gels and they concluded that this parameter plays a key role in the firmness of the final gel. Indeed, they observed that at 20 °C the storage modulus of gels was 30-fold higher than at 40 °C. Hydrophobic interactions were weakened with a decrease of temperature while hydrogen bonds were strengthened, which led to a more compact casein structure. As a consequence, the balance of inter and intra-casein micelles interactions changed with a decrease of temperature, leading in an increase of the storage modulus (van Vliet, Roefs, Zoon, & Walstra, 1989). Lucey and Singh (2003) suggested that since the rate of acidification is slower at lower temperatures, milk proteins have more time to establish new interactions, mainly hydrogen bonds. An alternative way of explaining this decrease in gel firmness, at high temperature, would be that fast acidification induces denser casein aggregates that fail to fully integrate into the new acid gel network (Phadungath, 2005). In our study, decreasing the rate of acidification by decreasing the GDL concentration does not improve the final firmness of gels (section 3.2.1) contrary to what observed with the decrease of temperature (Table 2). These trends are correlated in part to the high content of β-casein in dromedary milk as compared with cow milk (Table 1). This suggests an important role of hydrophobic interactions in maintaining dromedary casein micelle integrity and on the structuring of the dromedary milk protein network during acidification.

For the rest of the study, an acidification temperature of 45 °C was adopted to be close to the standard temperature of lactic fermentation.

3.3. Rheology of skimmed and reconstituted dromedary milk

The behaviour of skimmed dromedary milk (SDM) was compared with cow (RSCM) and dromedary (RSDM) skimmed spray dried milk reconstituted at the same protein level. The kinetics of acidification and the time-course of rheological parameters during acidification of the different milk samples are shown in Figs. 2 and 3, respectively, and summarised in Table 3. In addition, Fig. 4 shows how the storage modulus of the different milk samples changed over time as a function of the pH. Studies on acid gelation of dromedary milk reporting data on the evolution of storage modulus values are scarce. Attia et al. (2000a) reported a low final G′ of 16 Pa after a full 24 h of acidification of skimmed dromedary milk at 20 °C with a GDL concentration of 1.05% (w/v) but the evolution of rheological properties during acidification was not reported.

It has been reported that spray-drying is a non-denaturing method that preserves the quality of whey proteins (Oldfield, Taylor, & Singh, 2005). However, our study does highlight some differences in gelling behaviour between “fresh” and reconstituted dromedary milk (SDM and RSDM, respectively). First, it can be noted that the acidification of reconstituted skimmed milk occurs more slowly than for the initial non-reconstituted skimmed milk (Fig. 2). This trend could be due to the higher initial pH of reconstituted milk (6.55 ± 0.02) as compared with fresh skimmed milk (6.35 ± 0.04). It is well known that dromedary milk is richer than cows' milk in vitamin C (ascorbic acid) and its amount could be reduced after spray drying. Hence, we found higher pH values for reconstituted skimmed dromedary milk.

Although the general profiles of the evolution of rheological parameters during acidification are similar between reconstituted and fresh milk (Fig. 3B and 3C, respectively), the gel point for

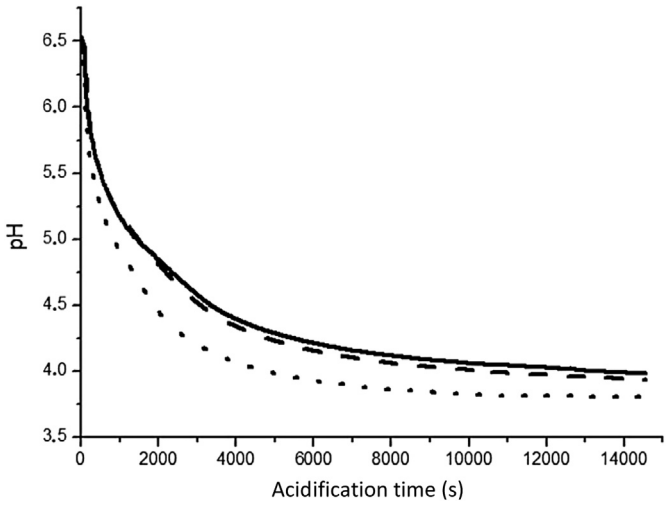

Fig. 2. Representative acidification kinetics of reconstituted skimmed cow milk (- - -), skimmed dromedary milk (•••••) and reconstituted skimmed dromedary milk (——). Acidification was carried out with 2.25% (w/w) glucono-δ-lactone at 45 °C.

reconstituted milk is reached at a significantly higher pH than for fresh milk (Table 3). Furthermore, after the gel point, reconstituted milk gel is significantly firmer for a given pH (Fig. 4). The differences observed in the gelling behaviour of reconstituted milk as compared with fresh milk is likely due to the larger diameter of casein micelles in reconstituted milk (Table 1). The improved gelling is likely attributable to the creation of interactions among casein micelles and whey proteins during the spray drying process. The influence of heat-induced protein aggregates on the rheological properties of acid gels for cow milk is well described in the literature (Andoyo, Guyomarc'h, Cauty, & Famelart, 2014; Donato, Alexander, & Dalgleish, 2007).

Here we demonstrated that reconstituted cow and dromedary milk have similar acidification kinetics when acidified under the same conditions (Fig. 2). However, the cow milk set significantly faster (Table 3): the gel point was reached in only 1292 s at a pH of 5.06. At this point, gelling pH and gel firmness are significantly higher than those obtained for skimmed dromedary milk (either reconstituted or not). Greater changes in gel firmness were observed after only 60 min from the gelling point (Figs. 3 and 4). During this time, the pH of each milk sample decreases drastically, and important physico-chemical changes occur. The results obtained in the present study for skimmed cow milk acid gels are in agreement with the findings reported in the literature (Lucey, Mishra, Hassan, & Johnson, 2005; McMahon, Du, McManus, & Larsen, 2009) and will be detailed and discuss in a later section (3.5).

After the full 3 h of acidification, the storage modulus of reconstituted cow milk gels was about 35 times higher than those of the two dromedary milk gels tested (Table 3). For cow milk, the increase of storage modulus is related to the intensive restructuring of the protein network that occurs before pH 4.4. Gel firmness was steady between pH 4.4 and 4.2, and then it continued to increase below pH 4.2 (Fig. 4). Unlike cow milk, dromedary milks have only one step for the G′ development (after pH 4.7; Fig. 4).

3.4. Microstructure of dromedary milk during acidification

Acid gelation of milk involves many structural changes. To study these micro-structural modifications, we analysed gels from each type of milk at different pH values by scanning electron microscopy (Fig. 5).

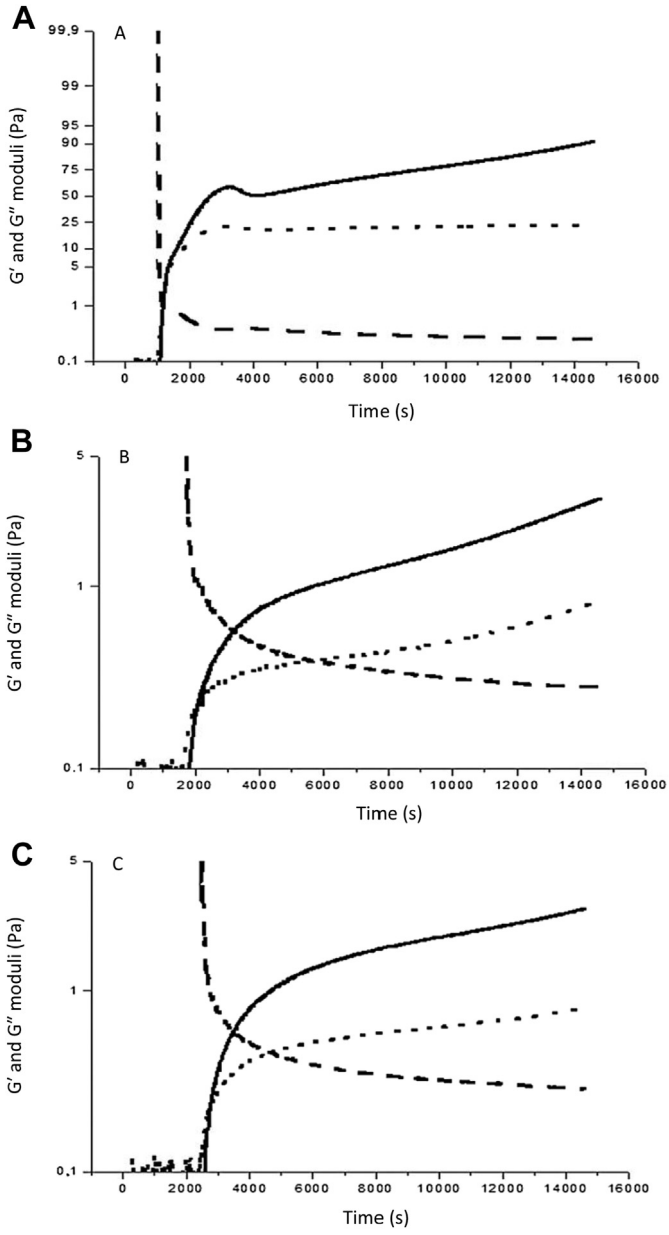

Fig. 3. Representative evolution of rheological parameters [storage modulus, G′ (——), loss modulus, G″ (•••••) and damping factor, tan δ (- - -)] during acidification of reconstituted skimmed cow milk powder (A), skimmed dromedary milk (B) and reconstituted skimmed dromedary milk powder (C). Acidification carried out with 2.25% (w/w) of GDL at 45 °C.

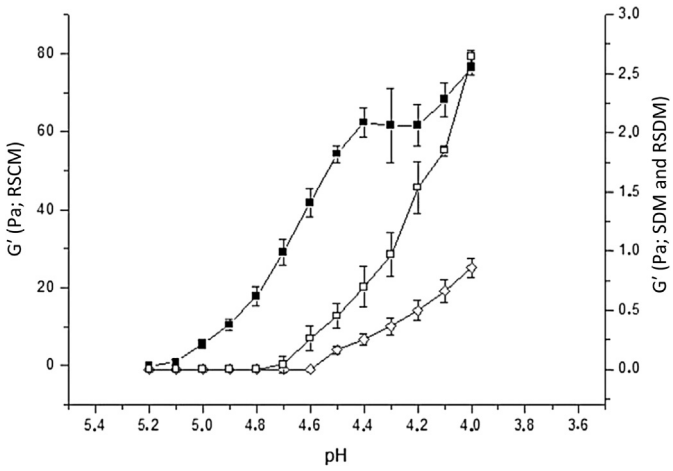

Fig. 4. Evolution of storage modulus (G′) of reconstituted skimmed cow milk (RSCM; ■, left ordinate), reconstituted skimmed dromedary milk powder (RSDM; □, right ordinate) and skimmed dromedary milk (SDM; ◇, right ordinate) during acidification to pH 4. Acidification carried out with 2.25% (w/w) of GDL at 45 °C; data are means ± standard deviation (n = 3).

Dromedary milks had larger casein micelles than cow milk at the initial pH (Fig. 5a), which confirmed our initial dynamic light scattering results (Table 1). At pH 5.5, caseins micelles from reconstituted cow milk aggregated and started to fuse together [Fig. 5b(iii)]. In dromedary milk, the linkage between casein micelles began at pH 5.5 but no fused structure was formed [Fig. 5b(i),(ii)]. Whereas there was a fused filamentous structure with large particles in cow milk at its gel point [Fig. 5c(iii)], dromedary milks had only a coarse protein network with just a few linked aggregates [Fig. 5c(i),(ii)]. As the pH approached pH 4 in cow milk there was a dense cluster covered with small whey protein aggregates [Fig. 5d(iii)]. In dromedary milk, however, there were still only non-merged small aggregates at this pH. It is likely that the larger casein micelles in dromedary milk have maintained their integrity during acidification [black arrow, Fig. 5d(i),(ii)]. Relatively few studies have focused on dromedary milk acid gelation. Attia et al. (2000b) suggested that dromedary milk casein micelles maintain their integrity until pH 5.5. They observed that approaching pH 5, dromedary casein micelles lose their integrity and the dromedary milk acid milk gel structure starts to appear. The pH range of 5.5 to 5 could be a transitional state from dromedary milk to dromedary milk acid gel (Kherouatou et al., 2003). It is likely that casein micelles kept a high mineral content, especially calcium ions, even at a high rate of acidification. Thus, they suggested that the insoluble mineral content of dromedary milk micelles was responsible for micelle resistance (preservation of the micellar structure) to acid gelation.

Furthermore, our results show no remarkable difference in the microstructure of fresh or reconstituted dromedary milk during acidification (Fig. 5). The fundamental differences observed between the microstructure of gels from cow and dromedary milks during acidification (Fig. 5d) are in agreement with their rheological properties (Table 3). During acid gelation, dromedary casein micelles aggregate but the fusion concerns only a few micelles, especially the smaller ones, contrary to what was observed for cow milk.

Table 3
Comparison of the characteristic parameters of acid gelation of dromedary or cow milk.[a]

Milk	Gel point			1 h after gel point		After 3 h acidification	
	Time (s)	pH	G′ (Pa)	pH		pH	G′ (Pa)
SDM	2225 ± 338[a]	4.47 ± 0.04[a]	0.20 ± 0.03[a]	3.975 ± 0.03[a]	0.92 ± 0.2[a]	3.83 ± 0.02[a]	1.74 ± 0.44[a]
RSDM	2810 ± 255[a]	4.65 ± 0.03[b]	0.20 ± 0.03[a]	4.20 ± 0.02[b]	1.1 ± 0.46[a]	3.99 ± 0.02[b]	2.43 ± 0.32[a]
RSCM	1292 ± 195[b]	5.06 ± 0.02[c]	0.78 ± 0.04[b]	4.22 ± 0.02[b]	64.7 ± 10.4[b]	3.94 ± 0.01[b]	83.6 ± 5.5[b]

[a] Comparisons were made at 45 °C using 2.25% (w/w) glucono-δ-lactone. Abbreviations are: SDM, skimmed dromedary milk; RSDM, reconstituted SDM; RSCM, reconstituted skimmed cow milk; Time, gelling time; G′, storage modulus. Values in a column followed by different superscript letters are significantly different ($p < 0.05$).

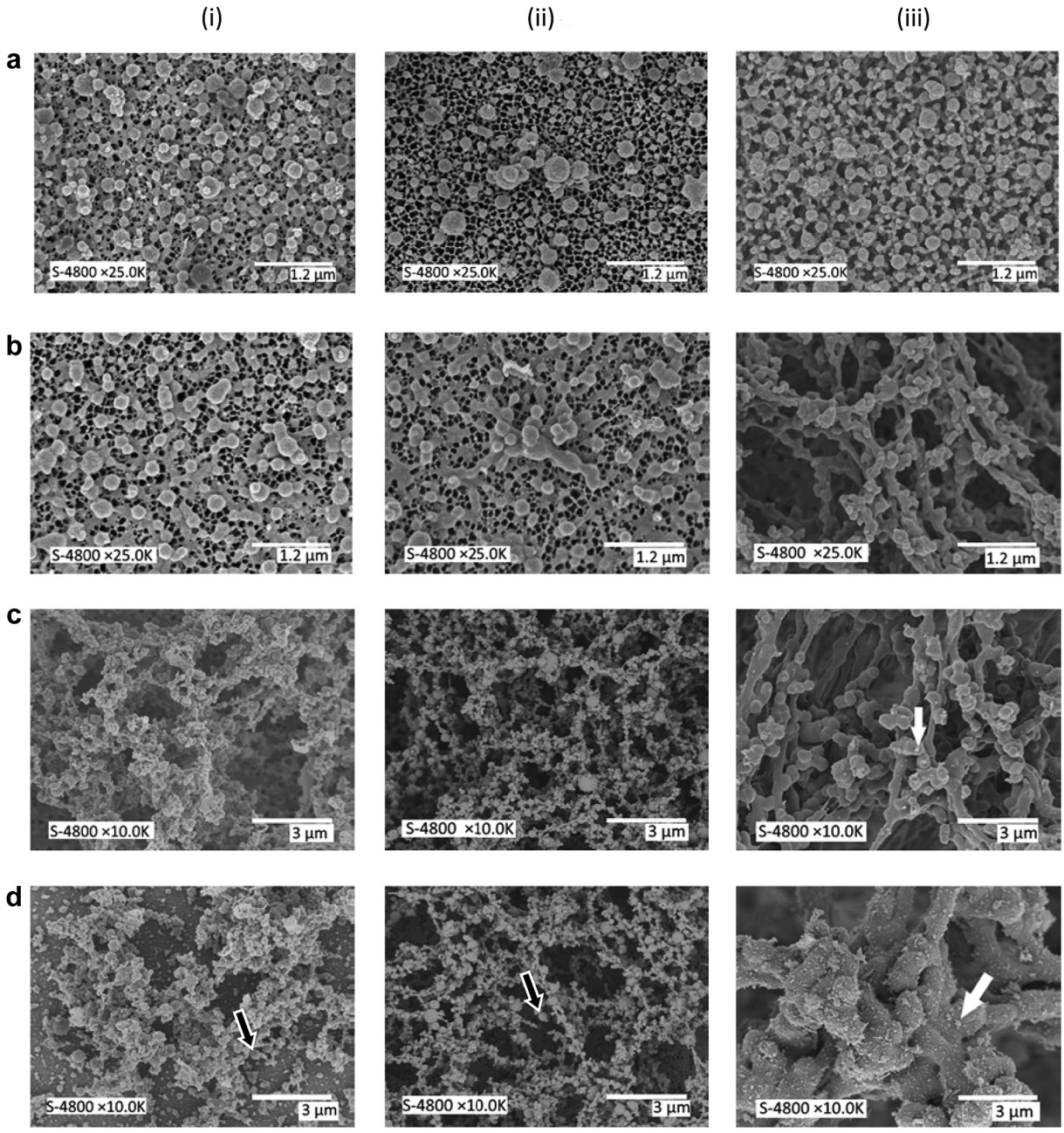

Fig. 5. SEM micrographs of the microstructure of (i) skimmed dromedary milk gels, (ii) reconstituted skimmed dromedary milk gels and (iii) reconstituted skimmed cow milk gels at different stages of acidification: a, initial pH [(i), pH 6.4; (ii) and (iii), pH 6.5]; b, intermediate pH (pH 5.5); c, gelling pH [(i), pH 4.47; (ii), pH 4.65; (iii), pH 5.06]; d, final pH (pH 4).

3.5. Gelling behaviour interpretation

Despite their similarities of composition, many physicochemical differences between cow and dromedary milk have been reported, especially in terms of micelle casein and whey protein composition (Al-haj & Al Kanhal, 2010). These differences likely influence the acid gelation process and explain the gelling behaviours observed. These hypotheses will be discussed below based on the current knowledge of acid gelation of cow milk.

The stability of casein micelles at neutral pH and their absence of coagulation are attributed to their global net negative charge and steric repulsion (Lucey, 2004). Overall, biochemical changes occurring during acidification of casein micelles leads to decreased electrostatic repulsion and increased hydrophobic interactions, which promote the aggregation and association of caseins into a new protein network (Lucey & Singh, 2003). It has been reported that the acid gelation process is deeply dependent on a balance between hydrophobic interactions and electrostatic repulsion (Morand et al., 2012). This balance is mainly dependent on the composition and properties of casein micelles. The caseins of dromedary milk have a lower isoelectric point than the homologous caseins of cow milk (Kappeler et al., 1998). In the same way, Wangoh, Farah, and Puhan (1998) observed an isoelectric point for dromedary milk casein micelles close to 4.3 versus 4.6 for cow milk casein micelles. These properties suggest that the casein micelles in dromedary milk maintain some negative charges, partial mineralisation, and stability at the gelling pH of cow milk that explains the lower gelling pH of skimmed or reconstituted dromedary milk observed as compared with reconstituted cow milk.

Furthermore, as already reported in several studies (Ereifej, Alu'datt, AlKhalidy, Alli, & Rababah, 2011; Omar, Harbourne, & Oruna-Concha, 2016), our results show that dromedary milk caseins have more β-casein (47%) and less κ-casein (3.5%) than does cow milk. In cow milk, the κ-casein content is negatively correlated to the casein micelle size, but positively to the firmness of acid gel. Furthermore, Gastaldi, Lagaude, Marchesseau, and Tarodo de la Fuente (1997) have shown that increasing κ-casein hydrolysis leads to an increase of whey separation and causes acid gel instability. Hence the low content of κ-casein in dromedary milk likely explains the higher size of casein micelles of dromedary milk as compared with cow milk (Table 1) and could play a role in the poor gelling ability of dromedary milk.

Mineral equilibrium, especially that of calcium ions, has been known to play an important role in the formation of the new protein network during acid gelation of cow milk and should also be considered important in dromedary milk gelation (Roefs, van Vliet, van den Bijgaart, de Groot-Mostert, & Walstra, 1990; Salvatore et al., 2011). Gaucheron (2005) reported that cation binding, essentially calcium, is directly related to the phosphoserine content of each casein following this order α_{S1}-casein> α_{S2}-casein> β-casein> κ-casein. The degrees of phosphorylation and glycosylation levels of caseins from dromedary milk are reported to be lower than those of cow's milk homologous proteins (Kappeler et al., 1998). However, the high β-casein content in dromedary milk could allow important interactions among proteins through colloidal calcium phosphate clusters. Thus, the richness in β-casein in dromedary milk likely helps maintain micelle integrity during acidification, through colloidal calcium phosphate bonds in the first step of acidification and/or more hydrophobic interactions at pH < 5.0.

Concerning whey proteins, dromedary milk lacks β-lactoglobulin while α-lactalbumin is reported as the major dromedary whey protein (El-Hatmi, Girardet, Gaillard, Yahyaoui, & Attia, 2007). At pH below 5, α-lactalbumin, in the absence of β-lactoglobulin, releases a part of its calcium content and switches to the molten state. In this state, more hydrophobic active surface and less electrostatic repulsion were observed (Zhang, Dalgleish, & Goff, 2004). Despite its similarities with cow α-lactalbumin, dromedary α-lactalbumin had a greater hydrophobic surface (Atri et al., 2010). However, its molten structure does not strengthen the new protein network of dromedary milk acid gel. Furthermore, it has been reported, for cow milk, that during acidification native β-lactoglobulin monomers self-associate to form octamer complexes in a pH range of 3–5. As the pH approaches pH 4, β-lactoglobulin reacts with α-lactalbumin to form whey protein aggregates (Harwalkar & Kalab, 1985) that promote the implementation of important electrostatic interactions with casein micelles inside the gel and enhance milk gel strength (Donato et al., 2007). Our results show that these aggregates (white arrows, Fig. 5) start to appear in cow milk at the gelling point and are strongly present at pH 4 (Fig. 5d). These large aggregates seem to be absent in dromedary milk acid gels even in spray dried dromedary milk.

4. Conclusion

This comparative study of acid gelation of cow and dromedary milk has allowed us to formulate some hypotheses to explain the poor capacity of dromedary milk to acid gelation. By testing different gelling conditions, we found that reducing GDL concentration and hence the rate of acidification did not improve the final firmness of the acid gels. However, we found a significant improvement of low acidification temperature on rheological gel properties around the gel point. At the gel point, a fused filamentous structure with large clusters was observed for cow milk. However, for skimmed or reconstituted dromedary milk the fusion consists only of smaller casein micelles.

For the skimmed and reconstituted dromedary milks, the gelling behaviours present only one step for the G′ development and it seems to be mainly controlled by a balance between hydrophobic interaction and electrostatic repulsion. This balance is dependent on the composition of casein micelles as well as their isoelectric points. Since dromedary milk has different casein proportions to cow milk and a lower isoelectric point, we suggest that intermolecular interactions inside dromedary casein micelles are stronger than those of cow milk during the acidification process. Therefore, during dromedary milk acidification, relatively few hydrophobic regions are exposed to the micellar surface and therefore balance equilibrium is not achieved. Thus, weaker hydrophobic interactions are established, and this makes the dromedary milk gels less firm. In addition, the lower κ-casein content and the total absence of β-lactoglobulin probably accentuate whey separation of dromedary milk and thus the acid gel's instability.

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